

Applicant : Silviu Itescu
U.S. Serial No.: 10/693,480
Filed : October 23, 2003
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In the Specification:

Please replace the paragraph beginning on page 117 at line 35 with the following rewritten paragraph:

DNA enzymes and RNA substrates: DNA enzymes with 3'-3' inverted thymidine were synthesized by Integrated DNA technologies (Coralville, IA) and purified by RNase-free IE-HPLC or RP-HPLC. The short RNA substrates corresponding to target DNA enzyme sequences were chemically synthesized followed by RNase-free PAGE purification and also made by in vitro transcription from a DNA template. Rat HBP23 cDNA and human PAG cDNA were amplified by RT-PCR from total RNA of cultured rat fetal cardiomyocytes and HUVEC, respectively, using the following primer pair: 5'TTTACCCTCTTGACTTTACTTTTGTGTGTGCCAC3' (forward primer) (SEQ ID NO:10) and 5'CCAGCTGGGCACACTTCACCATG3' (reverse primer) (SEQ ID NO:11). HBP23 and PAG cDNA were cloned into pGEM-T vectors (Promega) to obtain plasmid constructs pGEM-ratHBP23 and pGEM-humanPAG. cDNA sequences were verified using an automatic sequencing machine. 32P-labeled-nucleotide rat HBP23 and human PAG RNA transcripts were prepared by in vitro transcription (SP6 polymerase, Promega) in a volume of 20ml for 1 hour at 32°C. Unincorporated label and short nucleotides (<350base) were separated from radiolabeled species by centrifugation on Chromaspin-200 columns (Clontech, Palo Alto, CA). Synthetic RNA substrates were end-labeled with 32P using T4 polynucleotide kinase and incubated with 0.05%5uM HBP23 or scrambled DNA enzyme. Reactions were allowed to proceed at 37°C and were "quenched" by transfer of aliquots to

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tubes containing 90% formamide, 20mM EDTA and loading dye. Samples were separated by electrophoresis on 15% TBE-urea denaturing polyacrylamide gels and detected by autoradiography at -80°C. Primary rat fetal cardiomyocytes were obtained from Clonetic (USA) and grown in medium containing 2% FCS, 100ug/ml streptomycin and 100 IU/ml penicillin at 37°C in a humidified atmosphere of 5% CO₂. Cells were used in experiments between passage 6 and 8. Subconfluent (70-80%) rat fetal cardiomyocytes were transfected using 0.5ml of serum-free medium containing 0.05% 5uM HBP23 or scrambled DNA enzyme and 20 ug/ml cationic lipids (DOTAP). After incubation for eight hours cells were lysed using Trizol reagent (LifeSciences, CA) to isolate RNA for RT-PCR of HBP23 expression, as above.